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Review

Biological role of long non-coding RNA in head and neck cancers

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ABSTRACT

Aim: Head and neck squamous cell carcinoma (HNSCC) are one of the worst prognosis cancers with high mortality of patients. The treatment strategy is primarily based on surgery and radiotherapy but chemotherapy is also used. Every year the knowledge concerning HNSCC biology is updated with new elements such as the recent discovered molecules – long non-coding RNAs. Long non-coding RNAs are involved in regulatory processes in the cells. It has been revealed that the expression levels of lncRNAs are disturbed in tumor cells what results in the acquisition of their specific phenotype. lncRNAs influence cell growth, cell cycle, cell phenotype, migration and invasion ability as well as apoptosis. Development of the lncRNA panel characteristic for HNSCC and validation of specific lncRNA functions are yet to be elucidated. In this work, we collected available data concerning lncRNAs in HNSCC and characterized their biological role. We believe that the tumor examination, in the context of lncRNA expression, may lead to understanding complex biology of the cancer and improve therapeutic methods in the future.

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1. Head and neck cancers

Cancers of the head and neck area are prevalent in patients over 50 years old but they are becoming more common in young adults. Main features of head and neck squamous cell carcinoma (HNSCC) is poor—five-year survival (approximately 50%), depending mostly on the anatomical localization of the tumor. Oral cavity, oropharynx, hypopharynx, larynx and nasopharynx are the localization sites of squamous cell carcinoma.[1] The development of HNSCC is a multistep process of changes of normal mucosa and is often preceded by premalignant lesions such as leukoplakia.[2,3] HNSCC is mainly caused by exogenous factors such as cigarette smoking, alcohol consumption, unhealthy diet and HPV or EBV infections.[4,5] HPV positive tumors are molecularly and clinically distinct from HPV negative ones and HPV status is a significant prognostic factor.[1] Complete genetic characterization of HNSCC was described in the Cancer Genome Atlas Project. Analysis of 279 patients confirmed the already known changes in the genome and transcriptome and revealed the existence of previously undescribed molecular characteristics. Genetics and biology of HNSCC largely depend on the anatomical location of the tumor and the effect of carcinogenic factors. HPV-positive tumors are characterized by mutations in the PIK3CA gene, TRAF3 gene loss and E2F1 gene amplification. The second group, HPV-negative tumors, has mutations in CCND1, FADD, BIRC2, YAP1, CASP8 or HRAS and in other genes involved in the regulating cell cycle, cell death and NF-kB pathway. It has been shown that a common feature of HPV-positive and HPV-negative cancers are changes in TP63, SOX2 and PIK3CA genes. Characteristic features of HNSCC associated with smoking are mutations in the TP53 gene, inactivation of CDKN2A and changes in the genes associated with oxidative stress. It was noted that oral cancers, which have better responses to treatment, possess mutations in genes such as HRAS, PIK3CA, CASP8, NOTCH1 or TP53. Other types of HNSCC have changes in the NSD1, WNT and NFE2L2 pathway genes (especially cancers of the larynx). Regarding genetic background, HNSCCs were divided into four types of cancers: (i) basic (31% of cases), (ii) mesenchymal (27%), (iii) atypical (24%) and (vi) typical (18%).[6]

Biological knowledge of HNSCC continues to grow every year. Established by TCGA, molecular portrait of protein-coding genes is a helpful tool to test selected biomarkers. In previous report we indicated that several genes showed significant changes in expression in the HNSCC compared to healthy tissues based on cancer pathway analyses. And some of them can be used as predictive or prognostic factors.[7] Apart from protein-coding RNAs, more and more research focused on non-coding RNAs (ncRNAs). It was shown that ncRNAs—like miRNAs which can distinguish between cancer and healthy tissue—are present and dysregulated in patients' serum and can be used as a new, promising class of biomarkers.[8–11] In the era of next generation sequencing, the interest of ncRNAs is growing and new types of ncRNAs are discovered and thoroughly analyzed. One of the recently indicated molecules involved in the pathogenesis of HNSCC are long non-coding RNA (lncRNA).

2. Long non-coding RNA

Approximately 62% of human genome is transcribed, where about 30% of the transcripts correspond to exonic and intronic regions of protein-coding genes. The rest of them are mainly lncRNA in intragenic regions or antisense strands.[12] Long non-coding RNAs (lncRNAs) are a class of functional RNA molecules (consisting of at least 200 nucleotides) that are not translated into proteins and many of them are uniquely expressed in a specific type of tissue or cancer type.[13] It should be noted that some of RNAs possess dual functions of coding and non-coding molecules.[14] In the group of bifunctional RNAs, the following types can be distinguished: (i) lncRNA with small open reading frames (sORFs); (ii) coding and non-coding mRNAs as well as coding and non-coding isoforms (iii) by alternative splicing and (iv) with allele-specific features.[14] Expression of lncRNAs is regulated in a specific way, more than mRNAs, depending on cell types. In most cases they are evolutionarily conserved with regard to their function, secondary structure and regions of homology despite minimal overall sequence similarity.[15–17]

lncRNA biogenesis depends on lncRNA gene localization and share some features of protein-coding RNAs. The primary transcripts are proceeded by RNA polymerase II and they often undergo special processing events that were not observed in the case of mRNAs. The products of these processes are lncRNAs as well as mRNAs, short non-coding RNAs (pre-miRNAs, tRNA-like ncRNA) and different unstable forms of RNA molecules.[18] The intragenic lncRNAs (lincRNAs) can be encoded either in the sense or antisense orientation to host mRNAs and share hosts' promoters and enhancers.[19] However, some promoters of genes coding lncRNA and protein-coding RNAs are under distinct regulatory regime. Moreover, lncRNAs transcription is more sensitive to Dicer-miRNA-Myc circuit than protein-coding RNAs with Myc binding sites.[18,20] The lncRNA mechanisms of action depends on cellular localization. In the case of nuclear localization, they are involved in chromatin, transcriptional and RNA processing events; while in the case of cytoplasmic localization they are involved in mRNA stability or translation and they have influence on signaling cascades. Generally lncRNAs function as modulators of gene expression in cells in different ways: (i) signaling lncRNAs act as molecular signals due to specific biological events such as cellular stress leading to transcription activation of specific genes; (ii) decoying lncRNAs behave as modulators of protein factors such as transcription factors and chromatin modulators by binding to them and inhibiting their function consequently suppressing genes; (iii) guiding lncRNAs act as guides for specific ribonucleoprotein complexes and promote chromatin modification of target and (iv) lncRNA scaffolds modification of existing ribonucleoprotein complexes leads to transcriptional activation or repression, Fig. 1.[19–21] Another division of lncRNAs includes their regulative mechanism relating to location of controlled genes (i) close to transcription site of lncRNA (the same chromosome) named as cis-acting lncRNA (e.g. lncRNA-p21) and (ii) distant genes named trans-acting lncRNA (e.g. HOTAIR).[19]

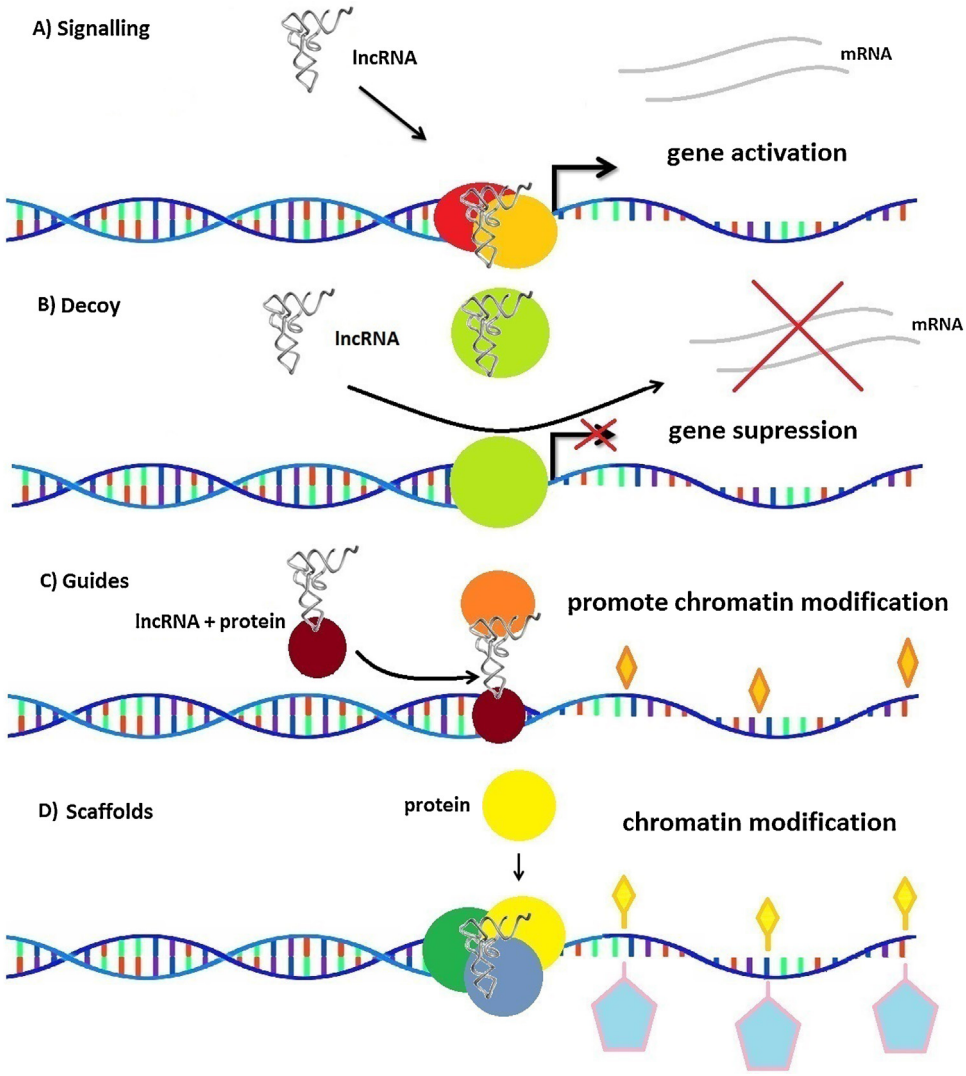

Fig. 1 – lncRNAs function as modulators of gene expression in cell. (A) Signaling-lncRNAs act as molecular signals due to specific biological events, such as cellular stress and lead to transcription activation of specific genes. (B) Decoying-lncRNAs behave as modulators of protein factors such as transcription factors as well as chromatin modulators by binding to them and inhibit their function leading to genes suppression. (C) Guide-lncRNAs act as guide for specific ribonucleoproteins complexes and promote chromatin modification of target genes. (D) lncRNA scaffolds modification of existing ribonucleoproteins complexes leads to transcriptional activation or repression.

It should be underlined that some lncRNAs encode miRNAs in their own primary or mature sequences. Moreover, some lncRNAs, named competing endogenous RNAs (ceRNAs), can possess multiple binding sites of specific miRNAs. ceRNAs protect target mRNA against repression by miRNA, e.g. H19 lncRNA acts as molecular sponge for the let-7 family.[21–23]

Like miRNAs, lncRNAs can be divided into two functional classes—suppressor and oncogenic. However, some of them can play dual functions depending on the type of cancer, for example BANCR or H19 lncRNAs.[24]

The role of lncRNAs in cancer is deeply examined and it is thought that they control important hallmarks of tumorigenesis. Processes responsible for local tumor growth (*in situ*) such as cell proliferation, regulation of cell death or angiogenesis are regulated by lncRNAs.[20] The role of lncRNAs is not only involved in local tumor growth but lncRNAs also have influence on invasion and metastatic processes.[14]

Yan et al. presented comprehensive characterization of lncRNA across 13 cancer types based on TCGA data. They concluded that dysregulation of lncRNA is highly cancer-type specific. More than 60% is uniquely expressed across cancers and only a few are dysregulated in all of analyzed cancer types.[25] HOTAIR, MALAT1, H19, BANCR, CCAT1 & CCAT2, UCA1 and FOXCUT are described as lncRNAs associated with cancer progression.[24] It is suggested that the imbalance of coding and non-coding transcripts of protein-coding genes may be associated with the cancerogenesis.[14] Among important events leading to dysregulation of lncRNAs are changes in somatic copy numbers of lncRNA genes, DNA methylation patterns in the promoter regions of lncRNA genes as well as cancer-associated SNPs (Single Nucleotide Polymorphisms).[25–27]

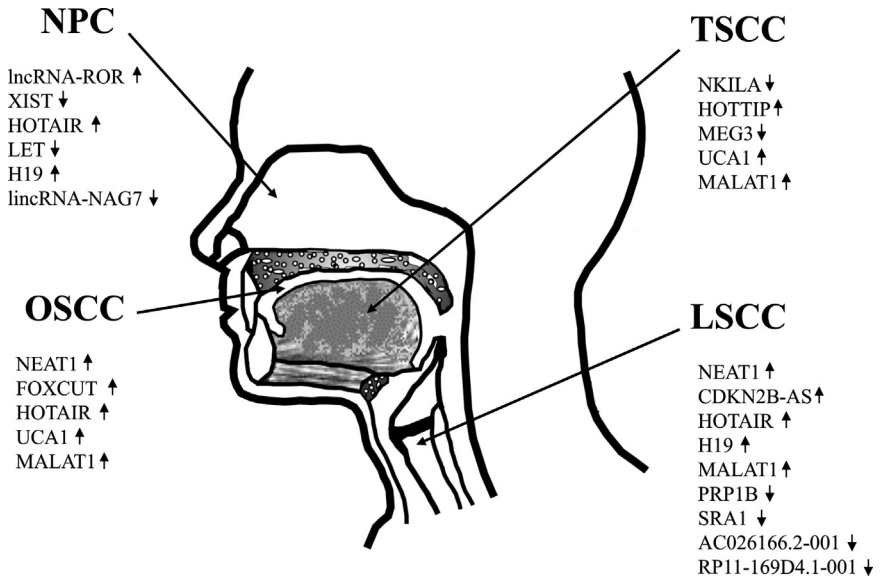

Fig. 2 – lncRNAs dysregulated in specific localization of HNSCC. (NPC, nasopharyngeal carcinoma; OSCC, oral squamous cell carcinoma; LSCC, laryngeal squamous cell carcinoma, TSCC, tongue squamous cell carcinoma.)

It is postulated that lncRNAs are one of the important elements maintaining anti-cancer drug and irradiation resistance and could be used as biomarkers.[28–30] Currently, many studies focus on the use of lncRNAs as predictors of survival in human cancers. However, many available studies are characterized by inaccurate results and poor methodology, which indicates a strong need of further research.[31]

3. lncRNAs in head and neck cancers

The global expression analysis of lncRNA presents profile of dysregulated lncRNA in cancer tissue of HNSCC. Four bioinformatic analysis of available data, three experimental microarray studies and one experimental next-generation sequencing revealed similarities and differences among various studies.[32–41]

The inaccuracies may be caused by differences in examined groups or samples (such as anatomical sites) reflecting genetic diversity. As showed in Fig. 2, the expression pattern of lncRNAs is closely connected with specific localization of HNSCC. However, some lncRNAs with strong prognostic ability of overall survival, disease-free survival or recurrence-free survival are proposed and they are independent of gender, organ site, tumor stage or TP53 status.[32,35,37,39] Some lncRNAs could also be used as the virus infection indicators[32] while others may serve as metastasis and disease progression markers.[34] The interactions between lncRNA and mRNA are indicated and connected with the regulation of transcription, apoptosis, cell growth and proliferation, migration and movement, cell differentiation, inflammatory and immune response, as well as metabolic and biosynthesis processes in the cell.[32–41]

lncRNAs regulate many hallmarks of cancer, such as the malignant phenotype, by regulation of epithelial-to-mesenchymal transition process (EMT) and influence on cancer invasion and metastasis.[24] Unfortunately, knowledge about lncRNAs involved in EMT process in HNSCC is still poor and there is no experimental evidence for most of lncRNAs. However, many dysregulated lncRNAs in HNSCC probably regulate transcripts associated with EMT or invasion/metastasis processes, which we indicated in Table 1. The clear evidence of EMT process regulation by MALAT1, lncRNA-ROR, lnc-KTCTD6-3, lnc-LCE5A1, NKILA and HOTAIR was indicated in HNSCC, and shown in Fig. 3.

It is well known that a tumor is not restricted to only one type of cancer cell, but various types with different phenotypes. One of which is cancer initiating cells (CICs), with unique genetic and behavior characteristics. Many studies characterize CIC populations in HNSCC and connect them with chemo or radioresistance.[42–46] It was indicated that lncRNAs can regulate pluripotency and differentiation of embryonic stem cells (ESCs) or induce pluripotent stem cells (iPSCs). They are also important regulators of adult stem cells.[19,47] One of the well-known lncRNAs implicated with pluripotency and tumorigenesis is SOX2OT. It was shown that SOX2OT positively regulates SOX2 and OCT4 genes—well-known markers of CICs. Moreover, expression of SOX2OT and SOX2 is increased in suspension culture of breast cancer cells compared to adherent ones.[48] It should be noted that hypoxia-related lncRNAs play important roles in tumorigenesis and could have important roles in CICs biology.[49] These results support the statement that lncRNAs are implicated in CICs populations in HNSCC. Unfortunately, no experimental evidence directly connects specific lncRNA and CICs in HNSCC.

The most studied lncRNAs in HNSCC are: HOTAIR, UCA1, LET, MEG3, MALAT1, H19 and NAG7. Their predicted targets, as well as other lncRNA indicated in HNSCC, are listed in Table 1. They are involved in many important cellular processes such as proliferation, migration and invasion, and apoptosis or phenotype regulation.

HOTAIR is a non-coding transcript of 2.2 kb that is transcribed from the *HOXC* locus at chromosome 12 but acts in

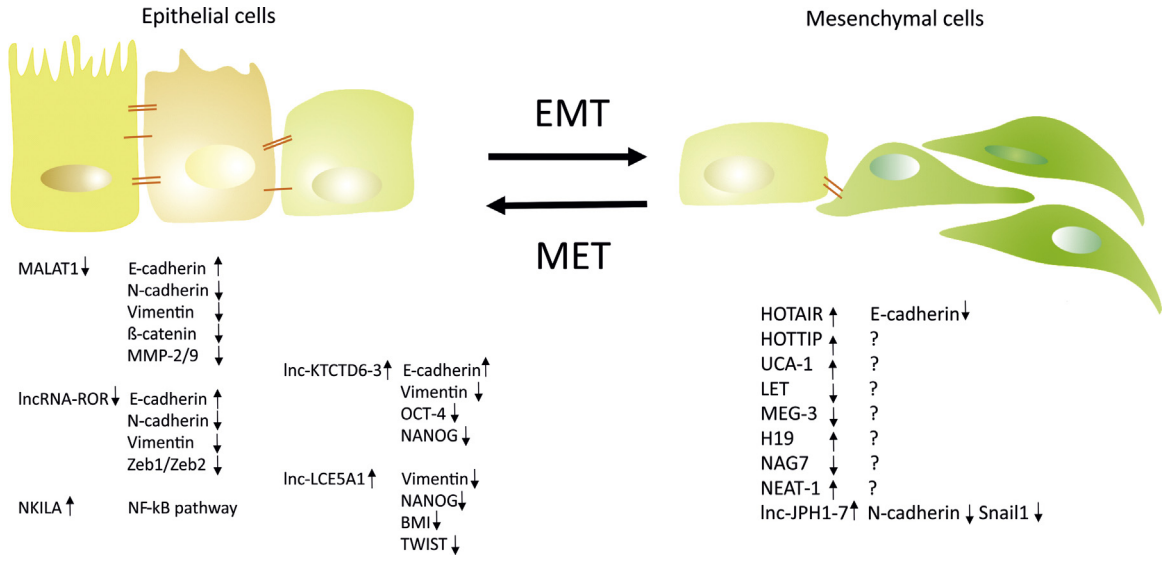

Fig. 3 – Role of lncRNA in epithelial-to-mesenchymal and mesenchymal-to-epithelial processes in HNSCC. Epithelial-to-mesenchymal (EMT) and mesenchymal-to-epithelial (MET) processes are one of the hallmarks of cancer progression. EMT process leads to change the cell phenotype, loose its polarity, cellular and extracellular junctions causing cell more motility. Probably some of lncRNAs have important role in this process.

transit at the *HOXD* locus at chromosome 2 thereby induces the transcription silencing.[50] The 5′-end of HOTAIR can bind chromatin-modifying complexes, whereas the 3′-end is able to bind histone demethylase I complex. Thanks to that, HOTAIR regulates the methylation of histone H3K27 site or the demethylation of H3K4me2 in genes related with the proliferation, apoptosis or metastasis of tumor cells.[51] The expression of HOTAIR was found to be increased in many subtypes of HNSCCs. Increased expression of HOTAIR was also associated with metastasis in breast and colorectal cancer patients.[52]

In laryngeal squamous cell carcinoma (LSCC) HOTAIR was found to be overexpressed compared to a noncancerous tissue. It is suggested that HOTAIR promotes the malignant progression of LSCC. High expression of HOTAIR was correlated with poor differentiation cancers, lymph node metastasis, resistance to apoptosis and more advanced clinical stages of LSCC.[50]

In oral squamous cell carcinoma (OSCC) a higher expression of HOTAIR compared to metastatic and non-metastatic tumors was observed. In addition, the level of HOTAIR correlated with lymph node metastasis,[53] tumor size, clinical stage and histological differentiation.[54] No differences were found in gene expression between males and females. Similarly, the age of patients did not have an impact on the level of expression of HOTAIR in OSCC.[53]

Wang et al. described the expression levels of HOTAIR and miR-21 in exosomes isolated from blood of LSCC patients. The expression of HOTAIR and miR-21 was increased and correlated with clinical stages, T classification and lymph node metastasis.[52] In addition, combined over-expression of miR-21 and HOTAIR can discriminate patients who are at the risk of developing LSCC with 94.2% of sensitivity and 73.5% of specificity.[52] For nasopharyngeal carcinoma (NPC) patients, HOTAIR is postulated as an independent prognostic marker for patients' progression and survival. However, it is also supposed to be more useful in advanced cases of the disease. Like in other HNSCC cancers, high expression of HOTAIR is connected with larger tumor size and advanced clinical stage. *In vitro* experiments indicated that HOTAIR is up-regulated in invasive NPC cell lines compared to low-invasive ones and knockdown of HOTAIR causes inhibition of cell proliferation, migration and invasion[55] and stimulation of cell apoptosis.[56] It is postulated that HOTAIR may stimulate tumorigenesis in NPC by pro-proliferation and pro-angiogenesis processes. The angiogenic function is manifested through direct transcription activation of VEGFA as well as by GRP78-mediated up-regulation of VEGFA and Ang2 expression.[56]

HOTAIR was also examined in OSCC cell lines. The expression was highly increased in OSCC Tca8113, UM-1 and CAL-27 cell lines compared to normal oral epithelial cell line.[54] Knockdown of HOTAIR in Tca8113 cells significantly reduced proliferation ratio by arresting the cell cycle and inducing apoptosis, which shows correlation of its expression with tumor size. The expression HOTAIR is negatively correlated with E-cadherin in patients' samples and cell lines. HOTAIR regulates E-cadherin through contribution of EZH2 and H3K27me3 binding with E-cadherin promoter. HOTAIR plays important role in carcinogenesis and it is not surprising that OSCC patients with over-expression of HOTAIR have poor OS and DFS.[54] Additionally, Kong et al. observed that depletion of HOTAIR induced mitochondrial-related cell death and involved pathways such as Bcl-2, BAX, Caspase-3, cleaved Caspase-3 and Cytochrome c. Injection of HOTAIR siRNA into tumors in a mouse model inhibited their growth.[57] Regulation of HOTAIR expression seems to be a good gene therapy candidate in HNSCC.

UCA1. Urothelial cancer-associated 1 (UCA1) long non-coding RNA is thought to participate in bladder cancer invasion and progression. Expression level of UCA1 is significantly enhanced in tongue squamous cell carcinoma (TSCC) and it is correlated with metastasis to lymph nodes.

Table 1 – Targets of lncRNAs described as deregulated in HNSCC.

lncRNA	Targets
HOTAIR	miR-124, miR-130a, miR-331-3p, β-catenin, Bmi1, CD11b, CD18, CD44, CD82, CD133, E-cadherin, HoxA1, HoxA4, HoxA5, HER2, KRT8, p16, p21, p53, PTEN, QKI, RBM38, SFN, WIF-1, MMP1, MMP2, MMP3, MMP9, MMP13, N-cadherin, Slug, Snail, Twist, VCAN, VEGF, Vimentin, Zeb1, Oct4
HOTTIP	HoxA7, HoxA9, HoxA10, HoxA11, HoxA13
UCA1	p21, CDKN2B, EP300, TGFβ-2, WNT6, AKT, CREB, p42, p44, ATM, Fas, PDGFB
LET	CASP3, BCL2, PCNA, Bax, p21
MEG3	CASP3, CASP9, p53, LC3-II, COL1A1, MDM2, α-SMA, CASP8, Dll4, Hes1, Iqgap1, MMP9, Timp1, Vegfa, Vegfr1, Wasl, BCL2, Bax, Cyto c, NF-kB
MALAT1	CCT4, CTHRC1, FHL1, ROD1, SLC26a2, TMEM20, AKT, p38, PI3Kp85α, β-catenin, CA2, E-cadherin, HNF4G, MIA2, RASSF6, ROBO1, ABCA1, ADAMTS12, AIM1, AKAP9, BMPER, CDCP1, COL6A1, CPM, CSF1, CXCL5, DRD1, GPC6, HMMR, LAYN, LPAR1, LPHN2, LY6K, MCAM, NNMT, PRKCE, Slug, Snail, STC1, Vimentin, Zeb1, Zeb2, ICAM, MMP9, PCNA, TNF-α, VEGF, Fibronectin, LTBP3, N-cadherin, p21
H19	let-7, miR-141, miR-200a, miR-200b, miR-200c, miR-429, miR-675, N-cadherin, Snail, Twist, Vimentin, Zeb1, Zeb2, Claudin1, E-cadherin, KRT19, KRT8, p53, Bax, IGF2, COL2A1, Lnsr, Lpl, Slug, CDH13, DICER, Hmga2, AKT, CDC25A, GSK3β
CDKN2B-AS1	ADIPOR1, C11ORF10, VAMP3, CARD8, Klf2, p21
GAS5	miR-21, CDK6, C-myc, ATG7, Beclin, Cyclin D1, E2F1, LC3-II, ADAMTS4, MMP13, MMP2, MMP3, MMP9, p21, p53, PDCD4, PTEN
linc-ROR	miR-145, miR-181, miR-205, miR-99, E-cadherin, Occludin, Fibronectin, N-cadherin, Vimentin, α-SMA, Zeb1, Zeb2, Nanog, Oct4, Sox2
XIST	X-chromosome, Atrx, Fgd1, Huwe1
FOXCUT	MMP2, MMP7, MMP9, VEGF, FoxC1
NEAT-1	HIV-1, ADARB2, EIF4G3, FP15737, OVC10-2, SH3PXD2A, F11R (JAM1)

The list of available predicted targets was taken from LncReg database (http://bioinformatics.ustc.edu.cn/lncreg/index.php).

Moreover, higher expression of UCA1 was detected in lymph node metastasis than in primary tumors. In cell culture of tongue squamous cell carcinoma, artificial over-expression of UCA1 promotes cells migration but has little impact on cell proliferation. These observations suggest that UCA1 might promote cancer cell metastasis and could be used as a prognostic indicator of lymph node metastasis in TSCC.[58] It was also found that UCA1 is expressed at higher level in OSCC.[53]

LET. In NPC patients, LET is significantly down-regulated in cancer tissue compared to normal ones and it is correlated with clinical stage, larger tumor size and invasion to lymph nodes. NPC patients with low expression of LET have poor recurrence-free survival and overall survival. In CNE2 cell line, enhanced LET expression inhibited cell proliferation and induced cell apoptosis by up-regulating the level of cleaved Caspase-3. Cells with over-expression of LET show reduced tumor growth in a nude mouse *in vivo* model. On the other hand, repression of LET promotes cell proliferation and inhibits apoptosis of NPC cells. It was found that LET is repressed by specific methylation of histone H3K27me3 on the LET promoter by enhancer of zeste homolog 2 (EZH2). Moreover, expression level of LET is inversely correlated with EZH2 in NPC tissues.[59]

MEG3. The expression of maternally expressed gene 3 (MEG3) in TSCC tissues is significantly reduced compared to nonmalignant tissue. The same phenomenon was observed in tongue cell lines SCC-15 and CAL-27 in comparison to human oral keratinocyte (HOK).[60] Expression of MEG3 is correlated with tumor size and its low level is associated with poor overall survival of TSCC patients. Reduced expression of MEG3 was also reported in hepatocellular carcinoma, gliomas[60] and OSCC.[53] On the other hand, it was indicated that expression of MEG3 correlated with miR-26a and higher expression of them is associated with better overall survival of TSCC patients. Moreover, expression of miR-26a and MEG3 is inversely correlated with methyltransferase DNMT3B. Over-expression of miR-26a or MEG3 in tissue cells and cell lines causes inhibition of cell proliferation, cell cycle arrest, and promotes apoptosis.[60]

MALAT1. Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is encoded inside the transcript of non-coding nuclear-enriched abundant transcript 2 (NEAT2)[61] and regulates gene expression as well as posttranscriptionally modification of primary transcripts.[62] It was shown that 3′ end of MALAT 1 plays a crucial role in the regulation of cell proliferation, migration and invasion.[62] It is positively correlated with clinical stage and serves as an oncogene in esophageal squamous cell carcinoma, glioma, renal cell carcinoma, lung and pancreas cancer.[61] Like in other cancers, MALAT1 is also over-expressed in HNSCC such as laryngeal,[62] tongue and oral squamous cell carcinomas[63]—especially in the case of metastatic TSCC.[64] In OSCC, MALAT1 is associated with poor prognosis and patients with low MALAT1 expression have significantly increased overall survival.

In oral cell lines inhibition of MALAT1 resulted in reduced cell invasion and migration, probably by regulation of MMP-2/9. Its over-expression induces a strong invasion capability by degradation of extracellular matrix in cancer cells.[63] Additionally, Fang et al. showed that MALAT1 modulates metastasis process of TSCC partially by the regulation of SPRR (small proline rich proteins) and genes associated with extracellular matrix and cytoskeleton regulation (LAYN, CCT4, CTHRC1 or FHL1). The knockdown of MALAT1 in SCC-25 and CAL-27 cell lines reduced cell migration ability and development of distant metastasis.[64]

Moreover, MALAT1 is associated with the epithelial-to-mesenchymal transition (EMT) process of OSCC *in vitro*. Knockdown of MALAT1 in Tca8113 and Tscca cell lines induces expression of E-cadherin and suppresses N-cadherin and Vimentin.[63] Also, reduction of β-catenin and impact on NF-κB signaling pathways were reported as a result of MALAT1 inhibition. It is well known that β-catenin plays a key role in cell invasion and angiogenesis by stimulation of MMPs and VEGF expression. The role of MALAT1 in cancer progression was also observed *in vivo*—its inhibition suppressed tumor growth of Tscca cell line injected to xenograft model.[63] All these findings indicate that MALAT1 is associated with cancer progression by

the regulation of multiple signaling pathways connected with cell proliferation, differentiation, migration, and invasion.

H19. The lncRNA H19 is a paternally-imprinted gene located in close proximity to the maternally-imprinted, insulin-like growth factor-2 (IGF-2) gene on chromosome 11.[65] The biological function of H19 has recently begun to be elucidated. It has been shown that the expression level of H19 is up-regulated in many cancer types, including esophageal cancer, breast cancer, colorectal cancer, gastric cancer, bladder cancer, and laryngeal squamous cell carcinoma.[66] In HNSCC, loss of imprinting of IGF2 or H19 may constitute a novel mechanism of the deregulation of growth-promoting genes in the development of these types of cancers.[67,68]

In LSCC tissue samples, H19 is up-regulated and its over-expression is correlated with the tumor grade, differentiation, neck nodal metastasis, clinical stage and poorer overall survival.[66] Knock-down of H19 inhibited LSCC cell migration, invasion, and proliferation. It was also proved that H19 and miR-148a-3p are negatively correlated and miR148a-3p is an inhibitory target for H19. However, over-expression of miR148a-3p did not affect the H19 expression level. H19 positively regulates the expression of DNA methyltransferase enzyme DNMT1 via miR-148a-3p inhibition.[66]

In NPC, H19 is over-expressed and correlated with enhancer of zeste homolog 2 (EZH2). Interestingly, higher levels of H19 were observed in poorly differentiated NPC cell line compared to the normal nasopharyngeal epithelial cell line.[65,69] Over-expression of H19 also promotes cell invasion and H19 knock-down significantly suppressed the invasive ability of NPC cell lines. Furthermore, H19 regulates EZH2 expression by interacting with miR-630 and inhibiting its activity.[69]

lincRNA NAG7 (LINC00312). It is a long intragenic non-coding RNA situated in the region of common allelic loss and it is associated with NPC. However, NAG7 transcript is also translated into protein—estrogen receptor repressor-10 (ERR-10) and probably functions as both coding and non-coding RNA.[70] LINC00312 is significantly down-regulated in NPC samples and its expression is positively correlated with lymph node metastasis and negatively correlated with clinical stage and tumor size.[71] Studies showed that up-regulation of NAG7 inhibits proliferation (G1/S arrest) and increases cell adhesion, motility, and invasion.[72]

Zhang et al. observed that clinical significance of LINC00312 expression level is connected with a lymph node status. In the case of patients without lymph node metastasis, the high expression of LINC00312 is associated with better DFS and OS. On the other hand, in patients with positive lymph nodes, the higher expression of LINC00312 is significantly connected with poor DFS and OS. Moreover, LINC00312 is negatively correlated with EBER-1 (non-coding RNA expressed in cells infected by EBV virus) and authors speculate that EBER-1 may regulate LINC00312 and have influence on cell behavior.[72]

Other lncRNAs. Unfortunately, the current knowledge about lncRNAs in HNSCC is still poor and ambiguous. Although there are comprehensive studies with regard to expression profiles of lncRNAs in HNSCC, they are not consistent. Moreover, there is little information about functional roles of lncRNAs in HNSCC. The available information about lncRNAs—which were indicated but very cursorily described in some studies—is presented in Table 2.

4. lncRNA in chemo- and radioresponse of HNSCC

It was presented that expression of lncRNA is changed after exposition to chemotherapeutic drugs and this phenomenon could maintain drug resistance of cancer cell lines.[38,62,82] Chen et al. showed that CDKN2B-AS1 (ANRIL), HOTAIR and MALAT1 were significantly reduced in Hep-2 and AMC-HN8 cells with the increasing concentration of cisplatin and paclitaxel. Moreover, the changes in the lncRNA expression continued for a long time and the most reduction was visible when cells were exposed to drug for 24 h. Unfortunately, authors did not connect changes in CDKN2B-AS1 (ANRIL), HOTAIR, and MALAT1 expression after drug exposition with some targets playing important roles in chemoresponse.[62] Comparison of lncRNA changes in paclitaxel-resistant CNE-2 and parental CNE-2 cell lines (nasopharyngeal) using NGS technology revealed 2670 known and 4820 novel lncRNAs. The most changed lncRNAs—n375709, n377806, n369241, n335785, Unigene6646, Unigene6644 and Unigene1654—were confirmed by qRT-PCR. Knock-down of n375709 in NPC 5-8F and 6-10B cell lines increased sensitivity to paclitaxel.[38] One of the well-described lncRNA regulating chemoresistant in NPC is lncRNA-ROR. Li et al. showed that lncRNA-ROR is up-regulated in NPC tissue and cell lines and regulates cell proliferation, cell cycle, apoptosis, migration, and invasion. Moreover, knock-down of ROR induces MET (up-regulation of E-cadherin and down-regulation of Vimentin, N-cadherin, Zeb1 and Zeb2). Application of cis-Dichlorodiamineplatinum (II) (DDP) to NPC cell lines induced up-regulation of ROR. Knock-down of lncRNA-ROR after incubation with DDP caused chemosensitivity of the cells through regulation of p53 pathway.[82] Only one study indicates lncRNAs as biomarkers to predict response to chemoradiotherapy. It shows that plasma circulating GAS5 is useful as a predicting biomarker. However, this study analyzed only a small and non-homogeneous group of patients, which might influence results.[78]

Up to date, one study describes the role of lncRNA in radioresistant NPC cell lines. Radioresistant NPC cell line was compared to parental one using NGS technology. In this study 310 up-regulated and 471 down-regulated known lncRNAs and 406 up-regulated and 1648 down-regulated novel lncRNAs were dysregulated in radioresistant CNE-2-Rs cell line. The up-regulated (n333177, n689, n375997, Unigene8485 and Unigene8588) as well as down-regulated (n376834, n381831, n375997, n341810 and Unigene3434 lncRNA) were confirmed by qRT-PCR. Three pairs of lncRNA-mRNA were indicated: n3739320-SLITRK5, n409627-PRSS12 and n386034-RIMKLB. However, after 4 Gy irradiation of two different radioresistant NPC cell lines, only slight changes of these lncRNAs-mRNAs pairs were observed in CNE-2-Rs cell line. In the case of the second radioresistant 6-10B-RS cell line, strong down-regulation of n373932 lncRNA and up-regulation of STITRK5 mRNA were observed and both of them are negatively correlated in NPC patients.[29] It should be noted that some reports indicated that CNE-2 cell line could be contaminated by HeLa cell line,[83] so the obtained results should be verified. The exact role of lncRNA in different subtypes of HNSCC regarding to radioresponse has remained unevaluated.

Table 2 – Other lncRNAs described as dysregulated in HNSCC.

lncRNA	Description	Ref.
NEAT-1	- The most abundantly expressed lncRNA in normal oral mucosa - One of the least stable lncRNA - Overexpressed in OSCC tissues but not found in patients' saliva - Overexpressed in LSCC, especially in advanced and neck nodal metastasis cancers - Regulator of proliferation, invasion, influence on cell cycle, inducer of apoptosis and tumor growth in xenografts - Regulator of CDK6 expression through up-regulation of miR-107	33,53,73,74
CDKN2B-AS1	- Up-regulated in LSCC - Inversely regulated by cisplatin and paclitaxel	62
HOTTIP	- Up-regulated in TSCC; associated with clinical stage, tumor size and distant metastasis - Higher expression significantly associated with patients' OS; an independent poor prognostic factor	75
RRP1B	- Down-regulated in LSCC	62
SRA1	- Down-regulated in LSCC - Unique modulator of steroid receptor transcriptional activity	62
AC026166.2-001 (MTCO1P5)	- Down-regulated in LSCC tissues and metastatic cervical lymph nodes - Low expression associated with poor prognosis	76
RP11-169D4.1-001 (LINC01537)	- Down-regulated in LSCC and significantly associated with cervical lymph node metastasis - Low expression correlated with poor prognosis - Potential role in tumorigenesis and migration of LSCC	76
GAS5	- Down-regulated in HNSCC and correlated with poor prognosis - Inducer of apoptosis process - Circulating GAS5 level as presumable predictor of patients' response to radical chemoradiotherapy with high sensitivity and specificity	33 77 78
lnc-JPHl-7	- Significantly associated with survival of both HPV+ and HPV- patients, advanced tumor stage, KMT2D mutation, TP53 mutation and 3p deletion - Influence on EMT process (N-cadherin, Snail1) and apoptosis (XIAP, DNA fragmentation)	35
lnc-KTCTD6-3, lnc-LCE5A-1	- Down-regulated in patients samples and cell lines - Influence on EMT process and cancer stem cells—up-regulated lnc-KTCTD6-3 increases E-cadherin and reduces OCT4, NANOG and Vimentin expression; up-regulated lnc-LCE5A-1 reduces NANOG, BMI, TWIST and Vimentin expression - High expression reduces cell migration and proliferation	33
XIST	- Up-regulated in NPC samples and NPC cancer cell lines - Connected with poorer OS, an independent risk factor for prognosis - Regulator of cell growth - Influence on up-regulation of oncogene E2F3 through 'spongeing' miR-34a-5p	79
FOXCUT	- Over-expressed in OSCC samples - Positively correlated expression of FOXCUT and FOXC1 - Regulator of cell proliferation, colony formation and invasion; influence on expression of MMP2, MMP7, MMP9 and VEGF-A	80
NKILA	- Down-regulated in TSCC; correlated with clinical parameters, metastasizing cancer and poor DFS and OS - Over-expression of NKILA reduces invasion and migration cancer cell ability through regulation of NF-kB pathway and influence on EMT process	81

5. Conclusions and future perspectives

Head and neck cancers are the most fatal. In clinical practice, patient's molecular profile of a tumor is not commonly used to personalized treatment. This molecular puzzle is now completed by a new element—long non-coding RNAs. However, only seven lncRNA profiling studies are reported and the changes in the expression during carcinogenesis process is still not clear in HNSCC. Moreover, these studies show many inconsistencies and, in our view, more independent screenings are needed. The open question is still the functional role of deregulated lncRNA in HNSCC. Biological role and targets of several lncRNAs were barely verified *in vitro* or *in vivo*. These described lncRNAs play important roles in cell proliferation, cell cycle, apoptosis, migration and invasion, angiogenesis, EMT process as well as probably in maintenance of CICs populations. However, this statement needs to be verified by more experiments, various models and clearer evidence, which show changes in specific cellular processes such as the regulation of CICs populations. CICs seem to arise in early steps of tumorigenesis and they are responsible for cancer development. However, CIC-related lncRNAs are still not clearly defined in HNSCC and they are an important challenge to

be described. It is well documented that aggressiveness and poor response to radio- and chemotherapy of HNSCC depends on distinct populations of CICs. The discovery of CIC-related lncRNAs may help to design the gene therapy strategies. The next problem that should be clarified is the role of lncRNA in radio- and chemical response. Only two screening experiments were performed in NPC cell lines to discover function of specific lncRNAs in radio- and chemoresistance, so the exact role needs to be verified more thoroughly. The use of relation of lncRNAs and cancerogenesis may dramatically change the HNSCC therapy and improve patients' outcome. Firstly, in diagnostics assessment of lncRNA, expression profile could describe the molecular portrait of a tumor and predict treatment strategies and their effectiveness. Secondly, the use of lncRNAs as gene therapy drugs is supposed to deplete CICs or reverse their phenotype consequently sensitizing cancer cells on the effect of the radio- and chemotherapy. However, this is still an intention. We should collect more information about lncRNAs and not forget the different elements of cancer biology.

Conflict of interest

None declared.

Authors' contributions

TK and KG have contributed equally to this work.

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